CREATINE SUPPLEMENTATION EFFECTS ON WEIGHT AND REPRODUCTIVE PERFORMANCES IN ADULT MALE RABBITS

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ABSTRACT

The objective of the present study designed to evaluate growth and reproduction performance when supplemented by creatine. Therefore, Twenty four adult male rabbits were divided into four groups (6 for each) randomly as following: Group 1 (standard) orally administered distilled water (1ml/animal) daily. Group 2: orally administration creatine monohydrate (1ml/kg BW) daily. Group 3: orally administration creatine monohydrate (2ml/kg BW) dissolved in 5ml distilled water by gavage daily. Group 4: orally administration creatine monohydrate (3ml/kg BW) daily. The results of final body weight revealed significant increase in treated groups as compared with control. The liver weights were statistically increment in creatine groups, while significantly decrement reported in relative weights of kidneys in creatine groups. While a significant decrease in the concentration of sperm, sperm motility % and viability of sperm respectively compared with standard group (G1) and a significant increment in abnormalities of sperm were recorded in creatine-administration groups as compared with standard group.

INTRODUCTION

Creatine formation interactions with carbohydrate can increase muscle creatine levels (by combining carbohydrates with creatine) more than creatine alone. creatine Supplementation (5 gm) with simple carbohydrates about 93 gm (for four times

/daily) for five days can elevat the levels of muscle creatine as much as 60% more than creatine alone (1).

In the liver kidneys, and pancreas, synthesis of creatine is performed with precursors of three different amino acids such as: glycine, arginine and methionine (2). The creatine formation are storage mainly occurred in skeletal muscle (about 95%), about 40% where it remains as free creatine or/and another as a creatine phosphorylation; moreover, it also correspond to 5% of the total found in the brain, liver, kidneys, and testicles (3, 4). Food supplies of creatine/day (about 1 gm) mainly through the animal products consumption such as fish and beef (creatine exogenous sources) in addition to synthesis of creatine in the body (creatine endogenous) (5, 6). The daily average requirement is 2 gm/day: 1 gm from diet and 1 gm from endogenous production (7 - 9).

Action of the Creatine's mechanism is based on trigger improvement that effects the metabolism of energy muscle, in which the ability to re-synthesize ATP by phosphorylation of creatine from adenosine diphosphate, thus 6-8 times increasing their deposits (9 - 11). Creatine supplementation have another beneficial effect to increase the muscle fibers size and also increase the mass of lean body, since there is an increased in protein synthesis and decrease in catabolism of protein. In the last, the creatine prevents damage of tissues, as it develops of cellular membrane stabilization mechanisms and maintenance of ATP molecules (4, 12, 13,14).

The present study aims to evaluate the toxicity of creatine monohydrate on body weight and reproductive functions in male mature rabbits.

MATERIALS AND METHOD

Experimental designation:

This experiment was done in animal house of the Veterinary Medicine College / Basrah University. Twenty four adult male rabbits were used and kept in cages (for acclimatization). The maintaining of rabbits (1.5-2 kg) under optimum condition (25±2) and 12/12 hours light/dark cycle throughout the experiment, with foods and tap-H_2O.

The mature male rabbits randomly were divided into four groups (6 rabbits/ group) as the following:

Group 1 (standard): Mature adult male rabbits orally administered of distilled water (5 ml/ animal/ daily) by orally gavage.

Group 2: Mature male rabbits were orally administration creatine monohydrate (1ml/kg BW) dissolved in 5ml distilled water by gavage daily.

Group 3: Mature adult male rabbits were orally administration creatine monohydrate (2ml/kg BW) dissolved in 5ml distilled water by gavage daily.

Group 4**:** Mature adult male rabbits were orally administration creatine monohydrate (3ml/kg BW) dissolved in 5ml distilled water by gavage daily.

Blood samples (10 ml) were collected via the cardiac puncture of anesthesia animals from heart by using 5ml syringe (sterile) putting in tubes without anticoagulant and then serum isolation by using centrifugation (3000 rpm / 15 min), stored it at -20ºC until analysis and then sacrificed the animals to take testes.

Hormonal analysis:

1- Testosterone ELISA Kit: a microplate enzyme immunoassay is a special kit cans quantitatively determination of serum total testosterone concentration (15).

2- Estimation for the Concentration of Follicle Stimulating Hormone (FSH) and Concentration of the Luteinizing Hormone (LH) (ng/ml): Estimation of FSH concentration by using an enzyme test kit (Human GmbH.53020 Wiesbaden. Germany Gesellechalf for biochemical and diagnostic mbH) (16).

3- Estimation characteristics of sperm epididymal: The epididymis of testis was removed, dissected and transferred into the petridish contain normal saline (5 ml) and the following as done:

A- Concentration of Epididymal sperm: The sperms count was done by using Neubauer hemocytometer chamber which uses for RBC and WBC counts according to the method of Robb *et al*. (17).

B- Percentage of Sperm motility: The individual motility of epididymal sperms was suggested by Chemineau *et al*. (18) that measured it depending upon the graduation basis as follows:

C- Abnormality of Sperm**:** The count of percentage of abnormal spermatozoa in the same slide that used for measurement of the viability of epididymal sperm using 200 sperms under a light microscope using 100X power.

Statistical Analysis

Statistical analysis was performed using SPSS version 22.0 for Windows. Independent "T" test analysis of variance with Tukey test was used to evaluate the differences among the two groups with regard to the study variables. Measuring of body and visceral weight are expressed as mean ± standard deviation (SD). A value of $P < 0.05$ was considered statistically significant.

RESULTS

I-Effect of creatine on BW and relative organs weight:

The data of result of BW demonstrated that no significant different in BW between creatine groups and standard group; while a significant ($p<0.05$) increase in final BW was showen in creatine groups (G 2, 3 and 4) as compared with G1. The weights of the relative organ of liver increased significantly ($p<0.05$) in creatine groups while kidneys relative weights were significantly ($p<0.05$) decreased in treated groups as compared with standard group (table I).

Table (I): Creatine effect on BW and relative weight of internal organs of male rabbits:

Parameters / Groups (n=6)	Initial BW (kg)	Final BW (kg)	Liver W. (gm)	Kidneys W. (gm)
G1 (standard)	1.10^{Aa} ± 0.12	1.30^{Cb} ± 0.08	42.25^{c} ± 1.70	9.17^{a} ± 0.15
G2 (1gm)	1.07^{Aa} ± 0.09	1.55^{Bb} ± 0.05	41.00^{c} ± 0.54	8.75^{b} ± 0.20
G3 (2gm)	1.05^{Aa} ± 0.06	1.53^{Bb} ± 0.075	44.00^{b} ± 2.70	7.58^{c} ± 0.05
G4 (3gm)	1.09^{Aa} ± 0.23	1.84^{Ac} ± 0.08	55.95^{a} ± 1.99	7.75^{c} ± 0.36
LSD	0.00	0.3 9	0 .76	0 .54
LSD within group	0.34	1.10	----	-----

The small letters indicate for values expressed in (Mean ±Standard Division) at ($p<0.05$) levels.

II- Creatine Effect on Concentrations of the Reproductive Hormones:

In the present study, the results showed that the creatine groups induced a significant decrement ($p<0.05$) in serum concentrations of LH, FSH and testosterone hormones as compared with the standard group (table II).

Table (II) Creatine effect on serum reproductive hormones concentration in adult male rabbits:

Parameters / Groups (n=6)	FSH ng/ml	LH ng/ml	Testosterone ng/ml
G1(standard)	4.17 ± 0.68 a	7. 18 ± 0.64[a]	7.27 ± 0. 32 [a]
G2 (1gm)	3.98 ± 0.47 a	4.75 ± 0.74 [b]	7.16± 0.70[a]
G3 (2gm)	2.56±0.61c	3.42± 0.93 [c]	5.25± 0.69 [b]
G4 (3gm)	1.82± 0.32 b	2.86± 1.00 [d]	4.40± 0.58 [b]
L S D	1. 24	1.10	1.50

The small letters indicate for values expressed in (M±SD) at ($p<0.05$) levels.

III- Creatine Effect on Characteristics of Epididymal Sperm:

The data in table (III) illustrated that creatine administration in a doses (1, 2 and 3 gm/Kg BW for 30 days) to the adult male rabbits induces a significant($p<0.05$) decrement in concentration of sperm, % motility of sperm and viability respectively as compared with standard group. Also a significant ($p< 0.05$) increment in sperm abnormalities were recorded in creatine-treated groups as compared with control.

Table (III): Creatine Effect on Epididymal Sperm Characteristics**:**

parameters / Groups (n=6)	Sperm Concent. ($\times 10^6$/ ml)	Motility of Sperm %	Viability%	Abnormality%
G 1(standard)	72.20 ± 6. 45[a]	8 2.70± 4.4 2 [a]	7 9.6 0 ± 3.3 9[a]	1 1.8 0 ± 3.0 1[a]
G 2 (1gm)	66.75±9.92 [ab]	80.50±7.21 [a]	81.00±5.04 [a]	12.75±3.84 [b]
G3 (2gm)	60.37±6.06 [b]	79.75±6.01 [a]	74.12±7.41 [b]	13.87±2.10[c]
G4 (3gm)	57.00±11.48 [c]	62.37 ± 11.43 [b]	73.62 ± 4.13 [b]	14.82 ± 4.31 [d]
LSD	7.65	6.43	3.76	0.86

The small letters indicate for values expressed in (M±SD) at ($p<0.05$) levels.

DISCUSSION

Methyl guanidine-acetic acid is a nitrogen amine referred to Creatine. The action mechanism of its based on the improvement the metabolism of the muscle energy, in which re-synthesize of ATP has the ability from the phosphorylated creatine. Also the supplementation of creatine produces other beneficial effect, such as: increased size of the muscle fibers and therefore the body mass. In light of these benefits, it is necessary to analyze its effects toxicity on important organs (liver and kidney). Extensively researched for Creatine supplementation has been supported to describe as one of the more effective dietary-supplements, also the creatine act as support strongly for creatine due to it has ability to safety role and lower risk for adverse effects or no effect on clinical safety and healthy (19).

The present study detected that administration of creatine monohydrate to the rabbits for 14-days at the dose (3 g/kg BW) resulted in significant elevation in final BW compared with standard group (table 1). These results are due to muscle water retention as a reason for creatine supplementation.

Kutz and Gunter studied (20) showed that Supplementation of creatine was taken for four weeks (thirty gm/day) for the beginning 2 weeks and then 15 gm/day for the last 2 weeks). Significantly increases were found in Total BW before and after the study for the creatine-group and doesn't significantly changes were found in fat of body % or intake calories daily in the creatine-group. Non changes were noted for the placebo-group. These results support many research that creatine-supplementation increment Total BW. There is not affected in Mean body fat% and caloric intake when creatine supplementation. Therefore weight-gain elevation in group of creatine administration may be due to retention of water.

This study showed increase in testosterone level in groups received creatine as than to standard-group (table 2). This data is in consistent with Merwe *et al*. (21) reported that resting concentrations of selected androgens after three weeks of creatine-supplementation in male rugby players. The hypothesize discus that the dihydrotestosterone ratio to testosterone that change with creatine administration. After seven days of loading creatine, or a further 14 days of creatine maintenance dose, serum Testosterone levels did not changes. The levels of dihydrotestosterone

increased about 56% after seven days of creatine that loading and remained about 40% above baseline after (14 days) of maintenance. The ratio between dihydrotestosterone: Testosterone which increased (36%) after seven days of creatine -supplementation and remained elevated more than 22% and after the maintenance dose. The significantly decrease in concentration of sperm, sperm motility, sperm viability and increase in abnormalities of sperm were seen in the current study as compared with standard group (table 3) may be explained by that, the Creatine that causes the muscles to consume large amount of water from the body that may deprive some amount needed for the seminal fluid. Therefore, high doses of creatine will reduce the quantity of the seminal fluid (table 4).

Studies evaluated these hypotheses reported that supplementing of creatine monohydrate to diet (with approximately 20 gm/day) for 2 to 7 days may increase total creatine levels in the content of muscles (about 10- 20%), and also the increment the creatine intramuscular level in the PC-form (20-40%) (22, 23, and 24). In addition, the studies that suggest the creatine-supplementation may have affect muscles of heart (25) and metabolism of skeletal muscle by speed up the ATP resynthesis rate during or / and following repeated bouts (fits) of high-intensity exercise (26). Theoretically this would improve repetitive capacity of sprint-performance.

Study the players of football were matched paired and assigned to supplement of creatine to diet eating for 28-days during agility to resistance training (eight hours/ week) with a Phosphagen hydrogen. Total BW increased significantly in the pure creatine monohydrate grouping while there is no differences were observed in the total body water % in control group (27 - 30).

آثار مكملات الكرياتين على الوزن والأداء التناسلي في ذكور الأرانب

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الخلاصة

في الدراسة الحالية، تم اخذ أحد أهم معايير الكفاءة الانتاجية : تقدير كفاءة النمو لجسم الحيوان وكفاءة الخصوبة للحيوانات. قسمت ذكور الأرانب البالغة (أربعة وعشرون / ٦ لكل مجموعة) إلى أربع مجموعات

بشكل عشوائي على النحو التالي: المجموعة ١ (السيطرة) اعطيت عن طريق الفم الماء المقطر (١ مل / حيوان) يوميا. المجموعة ٢: اعطيت الكرياتين أحادي الهيدرات عن طريق الفم (١ مل / كلغم من وزن الجسم) يوميًا. المجموعة ٣: اعطيت الكرياتين أحادي الهيدرات عن طريق الفم (٢ مل / كلغم من وزن الجسم) المذاب في ١٥ مل من الماء المقطر عن طريق المجرعة الفموية. المجموعة ٤: اعطيت الكرياتين أحادي الهيدرات عن طريق الفم (٣ مل / كغم من وزن الجسم) يوميًا. كشفت نتائج اوزن الجسم النهائية زيادة كبيرة في المجموعات المعالجة مقارنة مع السيطرة. زادت أوزان الاعضاء النسبية للكبد بشكل ملحوظ في المجموعات المعالجة بينما انخفضت أوزان الكلى النسبية بشكل كبير في المجموعات المعالجة. في حين أن انخفاضًا كبيرًا في تركيز الحيوانات المنوية تم تسجيل انخفاض في النسبة المئوية لحركة الحيوانات المنوية وصلاحيتها على التوالي مقارنةً بالمجموعة الضابطة وزيادة كبيرة في تشوهات الحيوانات المنوية في المجموعات المعالجة بالكرياتين مقارنةً بالمجموعة الضابطة.

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